

**Immature Stages of *Aganaspis pelleranoi* (Brèthes)
(Hymenoptera: Cynipoidea: Eucoilidae),
a Parasitoid of *Ceratitis capitata* (Wied.)
and *Anastrepha* Spp. (Diptera: Tephritidae).**

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Abstract.—The embryological-larval development of *Aganaspis pelleranoi* (Brèthes), a larval parasitoid of *Ceratitis capitata* (Wied.) and *Anastrepha* spp. is described and illustrated. *A. pelleranoi* possesses a stalked egg, eucoiliform first- and second-instar larvae, and hymenopteriform third- and fourth-instar larvae, followed by a prepupal and pupal stage. The duration of each stage at 25-26°C was as follows: egg, 3-4 days; first-, second-, and third-instar larvae, 2-3 days each; fourth-instar larva, 3-4 days; prepupa, 1-2 days; pupa, 9-14 days.

Aganaspis pelleranoi (Brèthes) is a parasitoid of *Ceratitis capitata* (Wied.) and several *Anastrepha* species (Diptera: Tephritidae) in Argentina (De Santis 1965), that attacks the final larval instar of these fruit flies (Turica and Mallo 1961). *Aganaspis pelleranoi* has also been reported from *Lonchaea* spp. (Diptera: Lonchaeidae) in Brazil (De Santis 1980).

From 1941 to 1945, *A. pelleranoi* was released as a control agent of fruit flies in Tucumán province, Argentina (Nasca 1973). In 1942 this species was introduced and reared in Perú for the control of *A. fraterculus* (Wied.) (Clausen 1978).

Ovruski (in press) carried out studies on host detection behaviour in this fruit fly parasitoid. Otherwise little is known of the biology of *A. pelleranoi*, and descriptions of the immature stages of this species have not been published. In fact very little at all is published on the development of eucoilid parasitoids of fruit flies, except that Clausen et al. (1965) briefly described the immature stages of *A. daci* (Weld) (= *Trybliographa daci* Weld), a parasitoid of *Dacus dorsalis* Hendel, the oriental fruit fly.

In this paper, studies on the life cycle and preimaginal development of *A. pelleranoi* are presented.

MATERIALS AND METHODS

Aganaspis pelleranoi was reared on last instar larvae of *C. capitata* in the laboratory as described by Ovruski (in press). The host larvae were reared on carrot/corn meal/yeast medium as described by Nasca (1977).

The life cycle and immature stages of *A. pelleranoi* were studied by exposing 20 to 30 *C. capitata* larvae to individual parasitoid females. The rearings and experimentation were conducted at 25-26°C, 70-80% RH, and 12:12 LD photoperiod. Host larvae and host puparia were removed at intervals of 24 h after parasitism and dissected in Insect Ringer's solution on depression slides. The parasitoid larvae were removed from each host puparium and preserved in 70% ethanol for later examination by light microscopy. Eight fourth-instar larvae were refixed in 4% glutaraldehyde and 0.2 M phosphate buffer, and dehydrated in 35-50-70-90-100% ethanol. Thereafter they were placed in 100% acetone. These specimens were critical point dried before examination by scanning electron microscopy (SEM). Descriptions are based on several specimens. Thirty-four eggs in different stages of development were mounted on slides and examined by light microscopy. Six first-, five

second-, and five third-instar larvae were mounted and examined by light microscopy. Eight fourth-instar larvae were observed with SEM and fifteen fourth-instar larvae were examined by light microscopy. Six females of *A. pelleranoi* of different ages were dissected in Insect Ringer's solution to observe the ovarian mature eggs. The terms used for morphological structure follow Kopelman and Chabora (1984) and Evans (1987). The drawings were made with a camera lucida. Data are presented as range and/or mean. Specimens, in ethanol and mounted, are deposited in the Miguel Lillo Institute Foundation, S. M. de Tucumán Argentina.

LIFE CYCLE

Females of *A. pelleranoi* deposited a single egg in the body cavity of the host larva and one parasitoid adult emerged from each host puparium.

Under laboratory conditions, the period from oviposition in the host larva to the emergence of a parasitoid adult varied from 25 to 27 days in males and from 26 to 30 days in females. Thus, males tended to emerge somewhat earlier than females. The egg stage inside the host larva lasted about 3-4 days. Observations of the postembryological development showed the presence of four larval instars. The first, second, and third instar each lasted about 2-3 days and the fourth instar 3-4 days (9-13 days in total). The prepupal stage lasted 1-2 days, and the pupal stage from 9 to 14 days.

EMBRYOLOGICAL DEVELOPMENT

The newly laid egg is 0.32 mm–0.56 mm long and 0.06 mm - 0.10 mm wide; the stalk is 0.42 mm - 0.73 mm long and the embryo is not yet distinguishable (Fig. 1).

24 h after oviposition inside the host larva, the parasitoid egg is 0.26 mm–0.48 mm long and 0.08 mm - 0.15 mm wide. The stalk is reduced in length, and the embryo, vitelline membrane and the chorion are well discernible (Fig. 2).

By 48 h after oviposition, the egg has lengthened and swelled (0.52 mm–0.66 mm long and 0.24 mm - 0.26 mm wide), and the stalk has decreased still more (Fig. 3). The embryo shows signs of body segmentation, a caudal portion and thoracic appendages.

After 72 h the parasitoid larva is completely

formed. The stalk disappears (Fig. 4) and the size of the egg is similar to the 48 h old egg. Eclosion of the first-instar larva occurs after 78-80 h. The chorion is broken through anteriorly by the mandibles and posteriorly by movements of the tail (Fig. 5).

LARVAL DEVELOPMENT

After hatching, the first-instar larva actively feeds on the internal tissues of the host. For this purpose, the mandibles are protruded from the oral cavity, although it was not possible to verify their use in feeding by direct observation. During this stage, the tail becomes less prominent and the head wider.

The second-instar larva appears on the fifth to sixth day after oviposition, when the host puparium is formed. The larva continues to feed inside the host pupa.

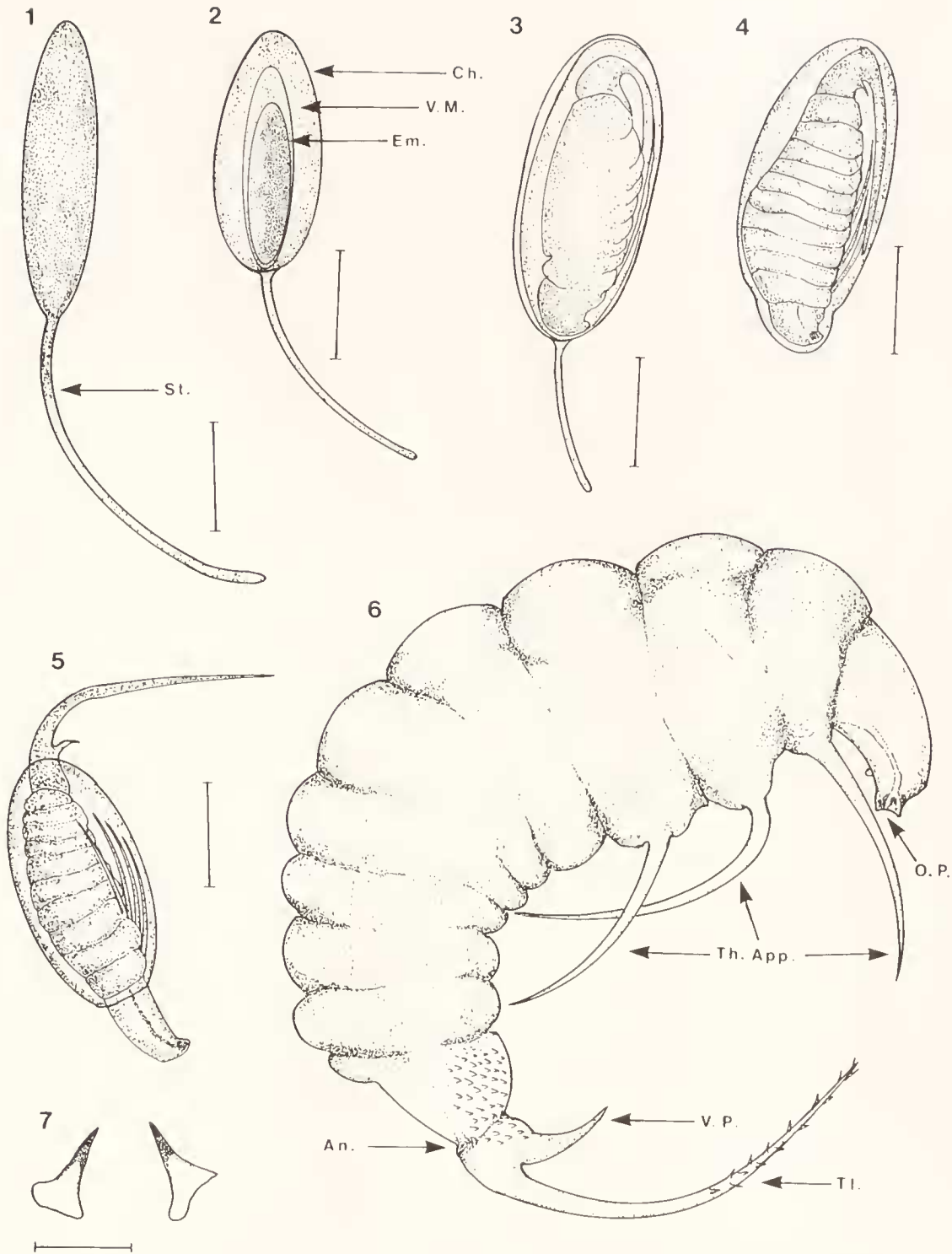
The third-instar larva appears on the eighth to ninth day after oviposition. In this stage the parasitoid larva partially emerges from the host pupa near the middle region, and it then feeds externally within the host puparium. The parasitoid larva occupies both internal and external positions in the host pupa and develops two respiratory mechanisms: cuticular respiration through submerged posterior parts of the body and tracheal respiration through prothoracic spiracles.

In the fourth-instar larva, which appears on approximately the tenth day after oviposition, feeding continues externally on what remains of the host pupa. The parasitoid larva is very sluggish and occupies 3/4 of the host puparium. After feeding is completed, the meconium is released. It appears as a dark crust in the posterior apex of the puparium (Fig. 14).

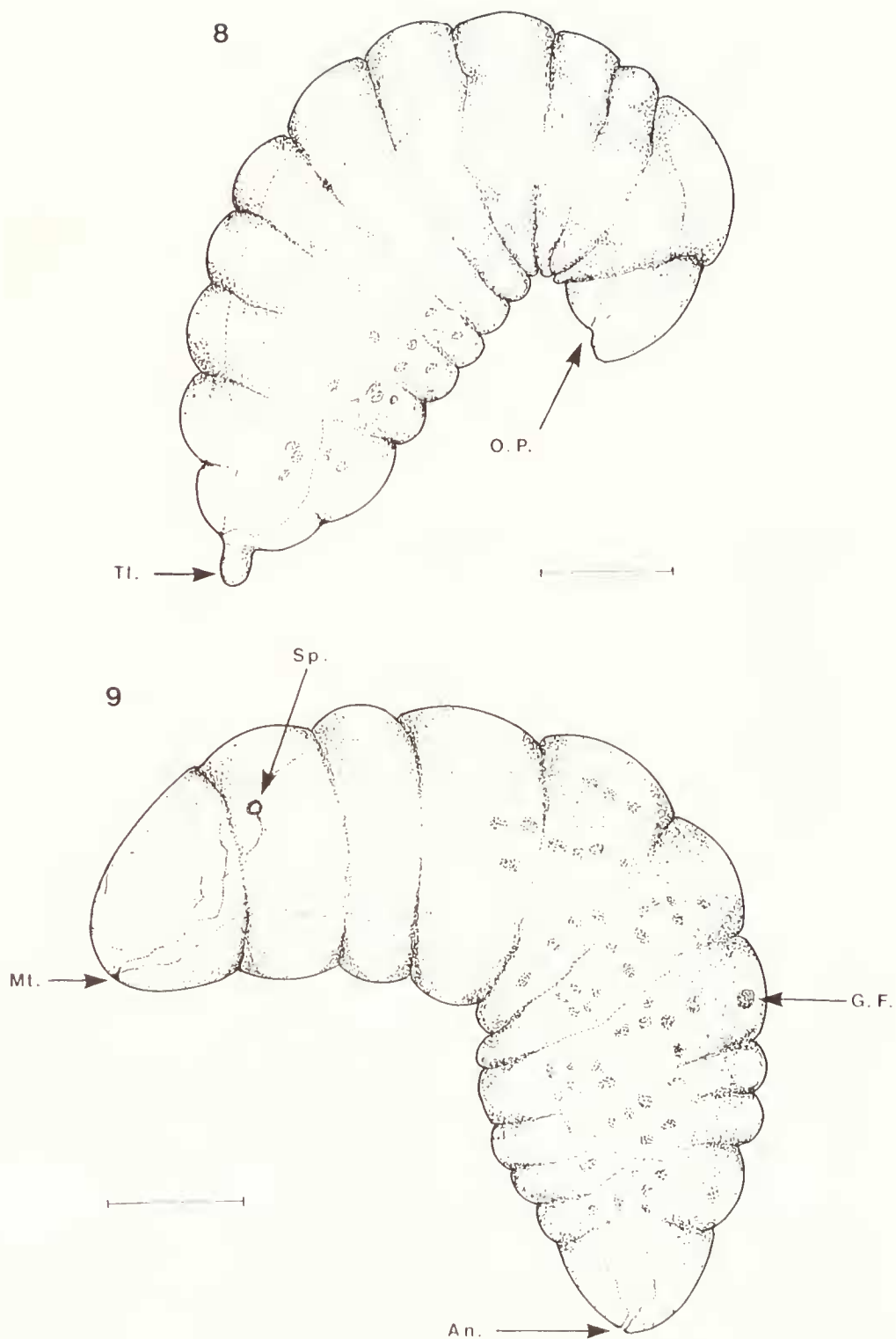
DESCRIPTIONS OF IMMATURE STAGES

Mature ovarian egg.— (Fig. 1.) Stalked; total length on average 0.60 mm; stalk 1.2 X longer than egg body, the latter portion 3.5 X longer than wide; chorion translucent and smooth.

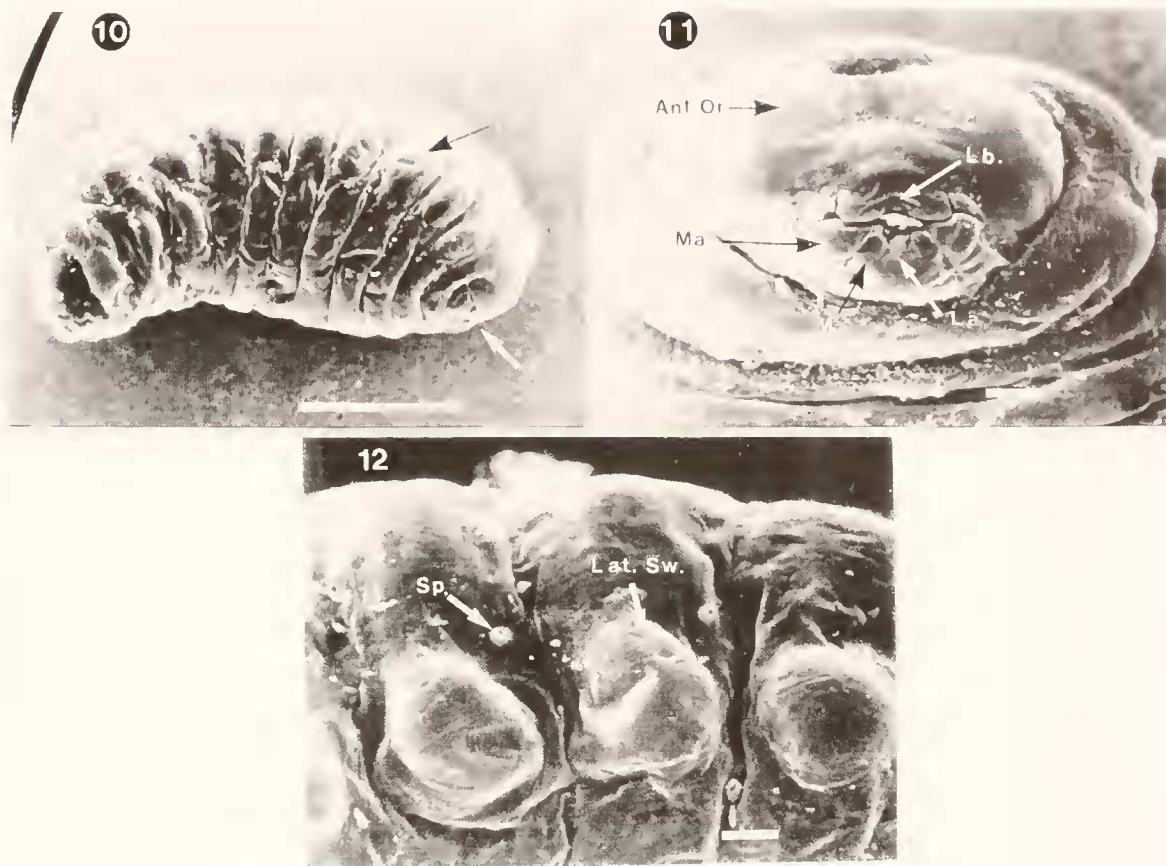
First-instar larva.— (Fig. 6, 7). Length 0.93 mm - 1.39 mm; 2.2 X longer than wide; eucoiliform; translucent; subcylindrical in shape with elongate distinct head; mouth surrounded by several oral papillae, inside with a pair of unidentate



Figs. 1-7. *Aganaspis pelleranoi*. 1, ovarian egg. *st.*, stalk. Scale bar = 0,12 mm. 2, egg at 24 h. after oviposition. *ch.*, chorion; *V.M.*, vitelline membrane; *Em.*, embryo. Scale bar = 0,12 mm. 3, egg at 48 h. after oviposition. Scale bar = 0,12 mm. 4, egg at 72 h. after oviposition. Scale bar = 0,12 mm. 5, egg at 80 h. after oviposition. Scale bar = 0,12 mm. 6, first-instar larva. *O.P.*, oral papillae; *Th. App.*, thoracic appendages; *V.P.*, ventral process; *Tl.*, tail; *An.*, anus. Scale bar = 0,12 mm. 7, mandibles of first-instar larva. Scale bar = 0,02 mm.



Figs. 8-9. *Aganaspis pelleranoi*. 8, second-instar larva. O.P., oral papillae; Tl., tail. Scale bar = 0,25 mm. 9, third-instar larva. Sp., spiracle; Mt., mouth; G.F., globular fat; An., anus. Scale bar = 0,32 mm.



Figs. 10-12. *Aganaspis pelleranoi*. 10, fourth-instar larva. *Lat. Sw.*, lateral swelling; *An.*, anus. Scale bar = 1,0 mm. 11, anterior view of head, fourth-instar larva. *Ant. Or.*, antennal orbit; *Ma.*, mandible; *Mx.*, maxilla; *La.*, labium; *Lb.*, labrum. Scale bar = 0,10 mm. 12, lateral view of body segment 6-8, fourth-instar larva. *Sp.*, spiracle; *Lat. Sw.*, lateral swelling. Scale bar = 0,10 mm.

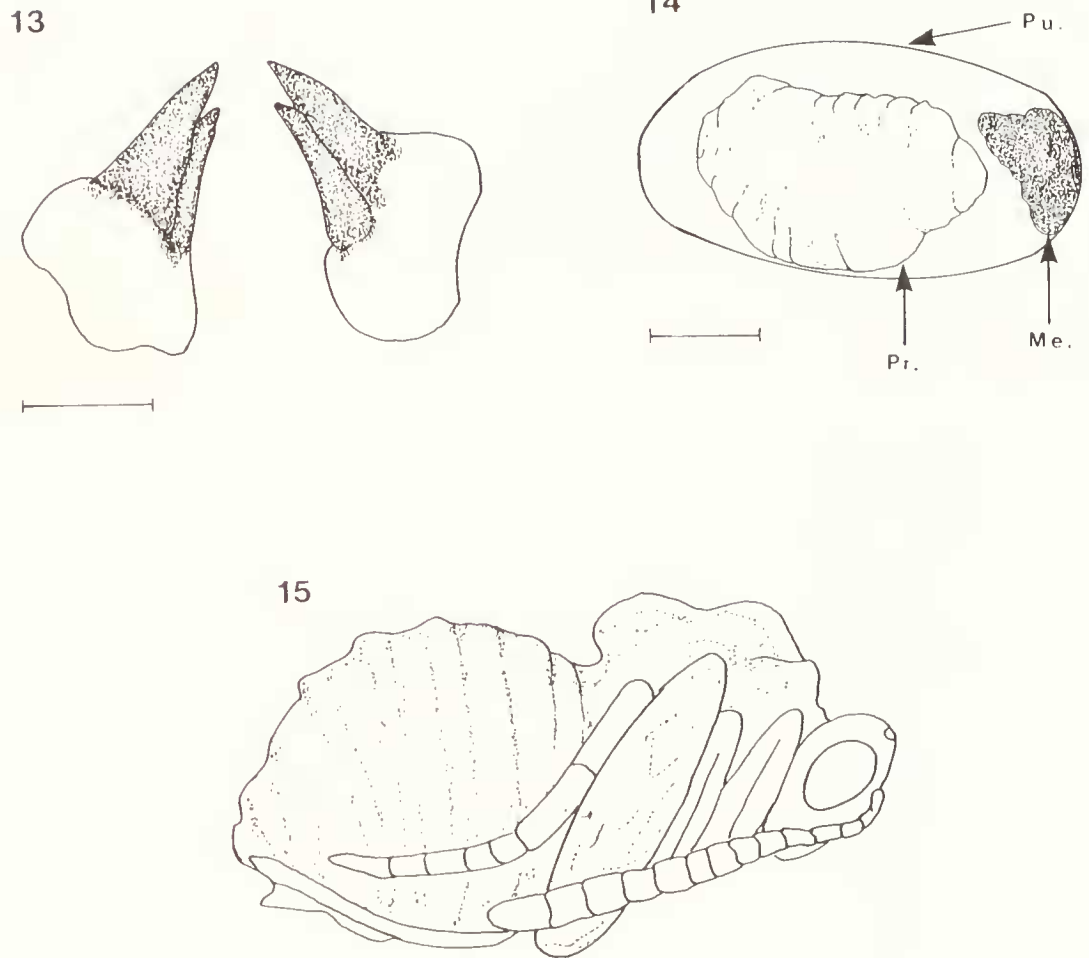
subtriangular mandibles (Fig. 7); ventral surface of head with a prominent tubular projection; gut easily discernible; each thoracic segment with a pair of long slender ventral appendages; seven well-defined abdominal segments; caudal segment with a long tail and short ventral process; tail bearing several small setae apically; ventral margin of 7th abdominal segment and basal end of caudal segment with scalelike ornamentation; tail 6 X longer than ventral process; anus opening dorsally.

Second-instar larva.—(Fig. 8). Length 1.53 mm - 2.31 mm; 2 X longer than wide; modified eucoiliform; whitish yellow with some white globular fat particles; cylindrical in shape with short and fleshy head; mouth with external oral papillae; unidentate mandibles; gut well discernible; without thoracic appendages; caudal seg-

ment with very short tail and without ventral process; anus opening dorsally.

Third-instar larva.—Fig. 9. Length 2.78 mm–3.45 mm; 3 X longer than wide; more typically hymenopteriform; yellowish with several white globular fat particles in thorax and abdomen; cylindrical in shape with slender posterior portion; broad head; small unidentate mandibles, difficult to discern; prothoracic segment with a single pair of spiracles; ten distinguishable body segments; brain and gut easily discernible; without tail and ventral process; anus opening ventrally.

Fourth-instar larva.—Figs. 10, 11, 12, 13). Length 3.48 mm–4.13 mm; 2 X longer than wide; hymenopteriform; whitish yellow with many small white globular fat particles dispersed throughout dorsolateral parts of thorax and abdomen; head



Figs. 13-15. *Aganaspis pelleranoi*. 13, mandibles of fourth-instar larva. Scale bar = 0,04 mm. 14, prepupa inside the host puparium. Pu., puparium; Pr., prepupa; Me., meconium. Scale bar = 1,0 mm. 15, pupa. Scale bar = 1,6 mm.

relatively large, subcircular in front view, without setae; antennal orbits large; prominent bidentate mandibles, 1.2 X broader than long basally, very dark apically (figs. 11 and 13); labrum 3.2X broader than long, with rounded lateral sides and median depression on apical margin; maxillae circular in shape and joined to labium; body very swollen; only eleven body segments discernible; except prothoracic and terminal abdominal segment, all segments with a pair of spiracles; each body segment provided with lateral voluminous swellings behind spiracles (Fig. 12); integument smooth and without setae; anus opening ventrally.

Prepupa.— (Fig. 14). Length 2.55 mm - 2.98 mm; width 1.18 mm - 1.56 mm; similar to fourth-

instar larva; body curved and reduced in size; occupying 2/3 of the host puparium.

Pupa.— (Fig. 15). Length 2.68 mm - 2.99 mm, initially white, later becoming dark; antennae 1,7 mm - 2.1 mm in length, extending to first abdominal segment.

DISCUSSION

Aganaspis pelleranoi produces a stalked egg, typical of the Eucoilidae. The transition from endoparasitic to ectoparasitic life has also been reported for other species of Eucoilidae (Jenni 1951; Wishart and Monteith 1954; Sychevskaya 1974; Kopelman and Chabora 1984). The embryological

and larval development is similar to other eucoilid parasitoids: *Trybliographa rapae* (Westwood) (James 1928; Wishart and Monteith 1954); *Hexacola* sp. (Simmonds 1952); *Hexacola* sp. near *Websteri* (Crawford) (Eskafi and Legner 1974); *Leptopilina bouvardi* (Barbotin et al.) (Kopelman and Chabora 1984); *Eucoila trichopsila* (Hartig) (Sychevskaya 1974) and *Kleidotoma japonica* Huz. (Huzimatu 1940).

Clausen et al. (1965) reported three larval instars in *A. daci*. This study proves the presence of four larval instars in *A. pelleranoi*. The first-, second-, and fourth-instar larvae described here are similar to the first-, second-, and supposed third-instar larvae of *A. daci* respectively. The third-instar larvae of *A. pelleranoi* is a transition between the eucoiliform type and the hymenopteriform type. This stage is distinguished by a single pair of open spiracles, in the prothoracic segment, as in the supposed third stage of *T. rapae* (Wishart and Monteith 1954), the supposed fourth stage of *E. trichopsila* (Sychevskaya 1974) and the supposed fourth stage of *L. bouvardi* (Kopelman and Chabora 1984). Further comparative work would be needed to confirm these apparent differences between species, as intermediate instars of apocritan larvae are notoriously easy to overlook.

Furthermore Clausen et al. (1965) stated that the first-instar larva of *A. daci* does not possess discernible mandibles, but the first-instar larvae of *A. pelleranoi* has a pair of well developed unidentate mandibles with their ends projecting from the oral cavity, similar to the first-instar larvae of *E. trichopsila* (Sychevskaya 1974).

Prominent bidentate mandibles in last instar larvae have also been reported for *A. daci* (Clausen et al. 1965), *Trybliographa Förster* and *Hexacola Förster* (Evans 1987).

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